This presentation will focus on two aspects of the motor design. Firstly the use of self-assembling components whose function is to co-localise the necessary motor functions. We demonstrate that a coiled coil template can be adapted in order to program the self-assembly of three different coiled coils from a solution of six peptides.

Secondly the role of linkers between components in controlling dynamics and hence both processivity and power strokes within our motor designs will be discussed. The use of cis-trans isomerization of azobenzene as a mechanism for driving conformational change within the motor will be demonstrated.

1. The Tumbleweed: towards a synthetic protein motor, EHC Bromley et al. HFSP J. 3, 204-212 (2009).
2. Tuning the performance of an artificial protein motor, NJ Kuwada et al. Phys. Rev. E 84(3) 031922 (2011).
3. Designed α-Helical Tectons for Constructing Multicomponent Synthetic Biological Systems EHC Bromley et al. J. Am. Chem. Soc. 131, 928-930 (2009).
4. Squaring the circle in peptide assembly: From fibers to discrete Nanostructures by de Novo Design. AL Boyle et al. J. Am. Chem. Soc. 134, 15457-15467 (2012).

1241-Plat

High Throughput Live-Cell FRET Binding Assay by Flow Cytometry

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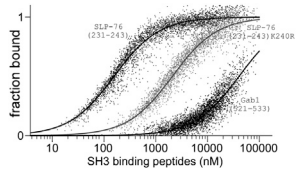
Ratiometric live-cell fluorescence resonance energy transfer (FRET) assays hold significant promise for measuring binding affinity of protein-protein interactions. Compared to conventional *in vitro* binding assays, live-cell FRET gauges interaction within a native regime that favors proper folding, permits physiological modulation of association, and obviates costly purification of proteins. Previously, our lab developed a live-cell FRET binding assay (three-cube method) for cell-by-cell epifluorescence and confocal microscopy (*Neuron***31**:973, *J Microsc***233**: 192). While accurate (*Nat Commun***4**:1717), this approach is comparatively slow and labor intensive, challenging high-throughput application. Here we transformed this live-cell FRET approach into the realm of flow cytometry, allowing full binding curves encompassing data from thousands of cells within seconds. We were able to get similar results between the new configuration and microscope-based setup. As an example, we characterized well-studied interactions between Venus-tagged SH3 domains of Gad and a suite of Cerulean-tagged binding peptides. Three such interactions are illustrated with a range of affinities, in close agreement with *in vitro* iTC measurements. This new flow-cytometry approach (3^3-FRET-HTS) opens new possibilities for profiling protein-protein interactions in the native context.



1242-Plat

Quantifying Cell-Surface Marker Expression Through Imaging of Transient Interactions

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Characterizing cells in terms of their surface-marker expression is an indispensable tool for cell biology, disease diagnosis, and drug discovery. Flow cytometry, the most commonly used method, though invaluable, requires exogenous labelling of cells, is not tractable for small sample sizes, and is limited in the number of unique markers that can be measured simultaneously. Recently, node-pore sensing (NPS) has emerged as a label-free method for surface-marker characterization. In NPS, the Coulter principle is utilized to measure the transit time of cells as they flow through a microfluidic channel functionalized with antibodies for multiple surface-markers of interest. Cells transiting functionalized regions of the channel that express the corresponding surface-marker have longer transit times when compared against a control region due to transient and specific interactions with the functionalized surface. In this manner, NPS can screen label-free for multiple surface markers at the single-cell level. The sensitivity of NPS is dependent on the ratio of the cell's volume to the volume of the channel, which limits the length of the channel and thus the number of possible unique markers. Furthermore, since the current through the channel is dependent on its total resistance, only one cell may be present in the channel at a time. Here, we show that these limitations can be overcome if the transit time is measured optically rather than electrically. Imaging the channel allows for tracking individual cells, which enables multiple cells to be measured simultaneously. Moreover, the total number of markers is only limited by the size and speed of the CCD camera that is used for imaging. We utilize this approach to measure the surface-marker expression profile of single MCF-7 and SKBR3 cells.

1243-Plat

Sorting Bacterium Cells Using Cell-Imprinted Polymer Thin Films: From Concept to Applications

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Cell imprinting is a recently developed technology that captures the structural and chemical information on the surface of cells on a polymer surface through template-assisted assembly of functional groups. A polymer is cured around template cells that are removed subsequently, leaving complementary cavities that not only spatially fit but also chemically recognize the target cells {*ACS Nano*, 6: 4314, 2012}. The cell-imprinted materials thereby function as artificial receptors, which are considerably less expensive to produce and more durable than natural receptors and could potentially be broadly used for cell sorting. One promising potential application is the detection of pathogens causing infectious diseases. However, the involvement of pathogens in the production of the imprinted material as well as in the cell capturing process brings occupational risk of infection. Most recently, we discovered that inactivated bacteria can be selectively captured by a polymer film imprinted with the bacteria inactivated in the same way, avoiding the use of live virulent bacteria; moreover, the inactivation strategies, especially those utilizing chemical reagents, resulted in better selectivity of capture than when living cells were used {*ACS Nano*, 7: 6031, 2013}. This inactivation process may have played two roles: (1) to eliminate the secretion of extracellular matrix, which helps expose the surface of the cells during imprinting; (2) to fix the cells, which helps preserve the structural and chemical information on their surface. Utilizing a cell-imprinted thin film, we are developing a rapid, culture-free, low-cost and high-sensitivity diagnosis for tuberculosis infection, which may fulfill the significant demand of effective and affordable diagnosis for tuberculosis in low-income countries. Also, we are developing an inexpensive cell-imprinted wipe for rapid detection of bio-contamination on solid surfaces, which may have broad applications in food safety control and public security.

1244-Plat

Patient-Specific iPSCs-Based Liver-On-A-Chip

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Successful advent of future medicine (e.g., personalized, preventive, predictive, participating medicine) will be strongly bonded to the availability of human-physiology-describing models. The combination of advanced researches in induced personalized stem cells (iPSCs) and the state-of-the-art microengineering is considered to create in-vitro models of organ functions in a near future; the first can generate patient-specific human cell lines, and the latter enables physiologically relevant microenvironments for recapitulating organ-level functions. Here we present a patient-specific iPSC-derived hepatocytes in an integrated microphysiological analysis platform (iMAP). The iMAP for iPSC-derived hepatocyte model is designed to culture the hepatocytes using endothelial-like physical barriers, which separate cell culture chambers and perfusion channels with Peclet number $<<1$. The physiological architecture supports enhanced hepatic functions of the iPSCs-derived hepatocytes, especially protein synthesis and drug metabolism. Compared to a conventional sandwich culture method, the liver-on-a-chip supports three-dimensional sinusoidal organization, enhanced junction protein and continuously refreshed media around the hepatic tissue to express higher albumin secretion and specifically enhanced drug metabolism (e.g., phenacetin is more metabolized to acetaminophen and N-acetyl-p-benzoquinone imine). Also, iPSCs-derived hepatocytes, by being matured from hepatoblast in the liver-on-a-chip platform, can increase the structural integrity due to avoiding dissociation of polarized hepatocytes and organogenesis-like tissue development in the microenvironment. The iMAP for iPSC-derived hepatocyte model will be tested for patient-specific pharmacokinetics/pharmacodynamics by involving various patient-derived iPSCs, and also other organ models will be integrated to estimate organ-organ interactions, which will provide various human-physiology-describing in-vitro models.

1245-Plat

A Biophysical Solution to Prevent Hemolysis Interferences

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In-vivo and *in-vitro* hemolysis, membrane rupture of red blood cells (RBCs) and release of their contents into blood plasma, is one of the major critical issues in medicine. For *in-vitro* blood-based molecular diagnostics, inhibitors (hemoglobin, potassium, mRNA etc.) released from RBCs due to hemolysis during blood plasma separation can cause serious difficulties in quantitative